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(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: MICROWELL PLATES

(57) Abstract

A multiwell plate where the wells have a large opening at the top and small nozzle hole in the base which is chosen so that a jet of liquid is emitted when a pressure pulse is applied to the surface such that by selecting a time for the pressure pulse a precise amount of volume in the well can be dispensed.

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TITLE: MICROWELL PLATES

Introduction

This invention relates to microwell plates.

High Throughput Screening (HTS) and Combinatorial Chemistry (CC) are key technologies in the search for new drugs. HTS addresses the screening of thousands of different compounds for a specific biological activity whereas CC addresses the manufacture of compound libraries by the parallel synthesis of different combinations of side groups on molecules of a particular structural class.

Many of the techniques used in both areas are based around the use of spatial arrays of reaction sites in the form of cells, microwells or immobilisation surfaces. For these approaches there is a requirement to move accurate volumes of liquid into and out of the cells in a short period of time.

One established approach is to use a microwell plate and a robot controlled micro-dispenser to fill and empty each well with its required chemicals in sequence. However, where the goal is to produce thousands of chemicals this approach is limited by the time taken to sequentially dispense to each well.

This patent application describes a system specifically designed for the parallel synthesis or assay of tens of thousands of compounds. The heart of the concept is a special well structure, called a **JetWell** in which the wells are arranged as a matrix within the flat plate as is conventional for microwell plates. The novel feature of this concept is that the wells are fabricated with large open areas at one end to accept liquid and integral nozzles at the other end from which liquid can be jet dispensed. These arrays might be made compatible with conventional 96 or 384 microwell plates but advantageously could be higher density (eg 60 x 40 or 120 x 80). The walls of the lower end of the cell may be coated with a

binding surface on which the compounds to be synthesised can be attached.

The design of the **JetWell** enables it to receive and deliver accurate micro volumes of fluid.

Dispensing of fluids required for the synthesis into each JetWell would preferably be made without contact of the dispenser to the liquid in the well by using a multinozzle ink-jet head firing into the large end of the wells. A multinozzle approach is necessary to ensure the dispense time for the complete array is short compared to the typical reaction time for each stage of the processing.

Liquid removal from the cells is achieved through the integral nozzle in the bottom of the well. During normal operation liquid is prevented from flowing out the cell through the nozzle by surface tension or even a physical plug. Liquid is ejected from the nozzle by applying pressure to the liquid in the wells causing it to jet cleanly out of the hole away from the lower surface.

The volume to be removed from the well can be controlled by the pressure applied and time for which it is applied. To achieve clean jetting of the fluid pressure typically in the range 0.1 to 10.0 bar gauge is used and the pressure is applied and removed rapidly to prevent any dribbling of liquid out of the nozzle. If a matching JetWell array is positioned beneath the first array transfer of an accurate volume of liquid from one array to the other occurs in a few milliseconds.

The approach provides an accurate system for the parallel dispensing into and from very small JetWell arrays. This enables the volume of the wells to be reduced and the number per plate increased thus greatly increasing the throughput of HTS or CC systems.

This patent application covers the fundamental concept and specific methods of implementing the individual components of the system.

<u>JetWell Array Structure</u>

The specific feature of a JetWell which is different from other forms of microwell plate is that each well has one or more nozzles in the base through which liquid in the well will jet when pressure is applied to the top surface.

The diameter of the nozzle controls the rate of liquid flow and is chosen to optimise for speed and accuracy. For example, if the pressure duration is controlled to 1 millisecond accuracy and the volumetric control requirement is 1 nanolitre the flow rate through the hole must be less than 1 microlitre/second. Assuming a jet velocity of 5 metres/second the nozzle would be around 50 microns in diameter which is comparable with that used in conventional ink jet printers.

The overall volume of each well depends upon the user requirements. A system might be designed to be compatible with conventional 384 well plates which have a well volume around 20 microlitres arranged on a 4.5mm pitch. However, there are considerable advantages in using larger arrays with smaller well volumes.

As an example a plate of similar size to a conventional microwell plate (either 96 or 384) with wells at 1mm pitch would contain a 96 x 64 array. Taking this size as an example the diameter at the top might be 0.8mm and the hole at the base 30 microns in diameter.

Figure 1 shows an example of the basic structure of the JetWell concept and Figure 1A is a detailed view, to an enlarged scale of part of that structure. This application, as well as describing the concept and example JetWell designs also addresses suitable manufacturing techniques for the plate and for the associated system components.

JetWell Plate Manufacture

Where implementations do not require holes below 0.1mm diameter in the base of the wells, conventional moulded manufacturing approaches may be used. However, as smaller holes are required then other approaches will be required. One approach is to use a moulded structure with a thinned section in the base through which a hole is drilled using a laser. Alternatively, a thin sheet with photolithographically defined holes, created

either by etching or electroforming, may be bonded to the bottom of the array.

Another approach is to form each line of wells by bonding two strips together with etched structures in them to form the wells. With this approach the complete array could be formed etched into the surface of a plate which is then cut into strips and laminated together to form the finished structure. Such a structure could be manufactured for example in glass using a low temperature glass enamel bonding.

Where very small wells are required then a single crystal silicon wafer substrate is appropriate with the wells formed by crystallographic etching which can be used to form the nozzle hole with a precision of a few microns.

Examples of these types of construction are shown in Figures 2a, 2b, 2c.

Dispense from the JetWell Plate

The JetWell concept has many wells in an array on a plate each with a nozzle hole in the base. It is possible to transfer a measured dose from all wells on a JetWell plate to a second receiver JetWell plate in one operation. This is achieved by placing the receiver plate directly below the plate containing the liquid to be dispensed and applying a pressure pulse to all wells of the top plate. Liquid is then jetted from each well on the top plate to the corresponding well on the bottom plate. To jet the fluid cleanly from the nozzle hole the pressure in the fluid must rise quickly and fall sufficiently fast to prevent dribbling from the nozzle. The dynamics of this are well understood from ink-jet experience and it is known that it requires rates in excess of 10^8Pa s^{-1} (ie 1 bar/ms) for typical dimensions.

Achieving this rate of pressure rise uniformly over the whole area of the plate is not trivial if contact with the liquid in the wells is to be avoided. One approach to achieving this is to use a piston arrangement over the whole area with the initial distance between the plate and the piston small compared to the lateral dimensions of the plate (eg 5mm spacing for a plate 60 x 40mm). This minimises the volume of air being compressed and the distance

in air for the pressure waves to travel.

The pressure rise of a few bar can then be achieved by moving the piston a few millimetres at speeds of a few metres per second. There are many options for implementing this motion. Where the areas are small, electromagnetic actuators are the simplest. However, for a plate area of 100 x 100 mm the force required to reach 10 bar is 10^4Newton's (~ 1 ton) which is more difficult to achieve using a simple solenoid drives.

To meet this requirement an impact actuator can be used. In this approach, the piston is sealed to the dispense JetWell plate using a compliant seal which permits sufficient travel to reach the desired pressure and then prevents over pressure by limiting the motion using a physical stop. The receiver JetWell plate is fitted directly below the dispense plate. The piston and the two JetWell plates are mounted together on a spring support. The back of the piston is then struck by a mass at a predetermined velocity. The mass striking the piston back moves the piston towards the plate until it reaches the stop. This compresses the air volume above the wells to the desired pressure very quickly. Both the impact mass and the plates continue to move compressing the support spring. The spring decelerates the assembly and then accelerates it back-up. Both JetWell plates are stopped at the original position but the piston is free to continue. As the piston and impact mass move away from the plates the pressure above the wells falls rapidly. The piston and impact mass are then decelerated, using viscous damping, to a stop. This apparatus is illustrated, by way of example, in Figure 3. It should be noted that there is a temperature rise associated with the rapid air compression which must be accounted for in the design.

Figure 3 shows two JetWell plates, each of the type shown in Figure 1, mounted in the impact actuator. JWL is the JetWell plate containing the liquid to be dispensed and JW2 is the JetWell plate into which the aliquots are to be dispensed. JW1 and JW2 are mounted rigidly together with JW1 above JW2. The piston plate PP is mounted with a small gap above JW1 on a compressible gas tight edge gasket EG. JW1, JW2 and PP are all supported on spring support SS.

In use, mass M is accelerated towards PP so that it impacts at a specified velocity. The piston plate PP and mass M move towards JW1 compressing the edge gasket (EG) until the motion stop (MS1) stops it at a preset gap. This raises the pressure rapidly and uniformly over all of JW1 to the pressure required to jet the liquid from the nozzles in the base of the wells. M, PP, JW1 and JW2 all continue to move down compressing spring support SS. The spring support SS oscillates forcing M, PP, JW1 and JW2 upwards after a time governed by resonant frequency of the spring/mass system. Upward motion of JW1 and JW2 is halted by the stop MS2 with PP and M continuing upwards by their inertia. PP and M are stopped by the stop MS3 at their starting position so that the pressure above JW1 returns rapidly to 1 bar.

The dispense pressure is a function of air volume and the distance to the forward stop and the dispense time is a function of the spring constants, masses and initial impact velocity. The technique therefore provides the ability to independently set time and pressure for dispensing over a wide range.

Where dispensing is required from a single well only then a small area piston pump can be located over the specific well. Similarly lines, or rectangular sections of the plate can be addressed by specifically shaped actuators. The use of a minimal volume positive displacement air pressure generator has important advantages as has the impact technique described to implement it.

Dispense to the JetWell Plate

The technique for dispensing from a JetWell plate can also be used for dispensing to a JetWell plate providing the same volume is required in each well. This is applicable in some, but not all instances. Where different amounts are required in each well, alternative approaches can be used.

Several approaches for this have been developed depending upon specific requirements.

One approach is to use the minimal volume pressure generator approach described previously with a patterned nozzle plate which addresses only those wells which are to receive the dose. This can be implemented by:

* Pre-fabricating a nozzle plate having the desired pattern of nozzles which is fitted to the dispenser when required. Nozzle plates could be selected from a library and cleaned for re-use

* A programmable nozzle array.

There are many possible implementations of programmable nozzle arrays including but not restricted to solenoid, piezo, mechanically and thermal drives for ball, plate, disc or poppet mechanisms.

However, implementation for nozzles in the sub 0.1mm diameter range with minimal dead volume and implementable in arrays of many thousand is a challenge. One approach is to use cone shaped nozzles which are produced as an array in a ferro magnetic material. The nozzle plate is removable from the pressure generating head. To programme the array to the required pattern, magnetic beads are placed in those nozzles to be closed. The magnetic attraction to the plate holds them in place whilst the plate is replaced in the dispense head. The nozzles are then filled with the liquid to be dispensed and a pressure pulse applied for the required time. The ball in cone arrangement ensures a good seal against the dispense pressure.

After operation the liquid is removed and the nozzle plate back-flushed to remove the beads before being cleaned for re-use. The magnetic beads may be disposable or reusable depending upon application. By using several plates which can be programmed whilst other operations are being performed the system will be rapid and flexible.

In other cases it may be necessary to dispense different volumes of a range of liquids to the wells. This can be achieved using the JetWell system in a pre-fill capacity. In this approach the JetWells of the dispense plate are prefilled with the volume and type of

liquids required using an accurate but not necessarily fast technique. The liquid is held in the wells by surface tension. When all wells have been filled with the predetermined volume of the appropriate liquid, the plate is ready for use. It is positioned over the reaction JetWell plate, and all the content of each well is jet dispensed by a pressure pulse as described previously.

This approach is most appropriate where it is necessary to make the transfer into the wells quickly but there is time between transfers to pre-load the dispenser. Care must be taken to avoid solvent loss by evaporation from the wells between filling and dispensing.

In some cases neither of these JetWell dispensing approaches will be appropriate. For these instances it may be necessary to use a precision dispensing head. The technique most suitable for this is based upon drop-on-demand ink-jet systems where the dose in each well can be set by dispensing a measured number of drops.

The most rapid dispensing would be achieved by having a two dimensional array of nozzles to match the plate. However, this is difficult to construct and hence expensive. The preferred approach is to use a linear array of nozzles which is scanned across the plate. Commercially available ink-jet heads are unlikely to be appropriate, mainly because of the pitch between nozzles and their choice of materials, and a specific implementation is therefore needed. The features of the head which are required are:

* The dead volume of the system must be minimised to avoid wastage of reagents which in some cases may be expensive

* All material in contact with the fluid must be inert to a wide range of acids, alkalis and solvents. Preferably it should be restricted to silicon dioxide, stainless steel, and inert polymers

* The system must be easy to fill and to flush as it must be used many times with a wide range of fluids

* The nozzle diameter is chosen to match the well size. Typically the drop size is selected to be in the range 10^{-3} to 10^{-4} of the well volume

* Each nozzle is operated independently in a drop on demand mode. Typical delivery rates are in the range 10^3 to 10^4 drops per second

* Actuation will preferably be by a thin piezo layer bonded to a silicon or silicon dioxide membrane.

For high density arrays the piezo drivers may be integrated on to the head. Alternatively the head would have gold contacts deposited enabling it to make electrical contact to off head drivers.

The application lends itself to implementation of the head using silicon crystallographic etching techniques to form the structure and either silicon to glass or silicon to silicon bonding for assembly.

Either top shooter or side shooter forms could be implemented at typical pitches required ($>$0.5mm).

<u>Calibration</u>

The precision of dispensing achievable either by pressurised jetting or by drop on demand devices depends upon the accurate control of a range of parameters. Where the nozzles become very small it is difficult to achieve precision from the accuracy of manufacture alone. However, by reasonable control of the operating parameters reproducibility can be excellent.

The best approach for achieving precision is therefore to include a technique for the calibration of each device prior to use. Thus, for example, for JetWell plates there may be a distribution of nozzle diameters coupled with a pressure variation which leads to different volumes being dispensed for each well. If a quantative assay is being performed

on part-samples from each well, volume variation would influence the result.

The system could be calibrated before use by dispensing a reagent which can be used in a simple standard assay test for volume. This for example could be a colour change reaction which is convenient for automated measurement.

The calibration would be used to correct the results of the real assay rather than to control the volumes dispensed.

The implementation of automated calibration is an important technique for achieving high precision for multinozzle microdispensing systems.

CLAIMS

1. A multiwell plate where the wells have a large opening at the top and a small nozzle hole in the base.

2. A multiwell plate where the diameter of the small hole in the base is chosen so that a jet of liquid is emitted when a pressure pulse is applied to the surface such that by selecting a time for the pressure pulse a precise amount of the volume in the well can be dispensed.

3. A multiwell plate as in (1) which has immobilisation coating on the walls for the purpose of synthesis or assay.

4. A multiwell plate as in (1) in which the nozzle is fabricated by moulding, laser drilling, photolithographic processes, or by etching.

5. A multiwell plate according to claim 1 which is fabricated by stacking layers together which have etched structures which form the wells.

6. The use of an air pressure to eject measured volumes of liquid from the nozzles in the base of the wells described in (1).

7. The generation of the air pressure pulse by the movement of a piston plate in close proximity to the surface of the multiwell plate.

8. The generation of the movement of the piston used in (6) by electrical, hydraulic or mechanical impact means.

Fig 1

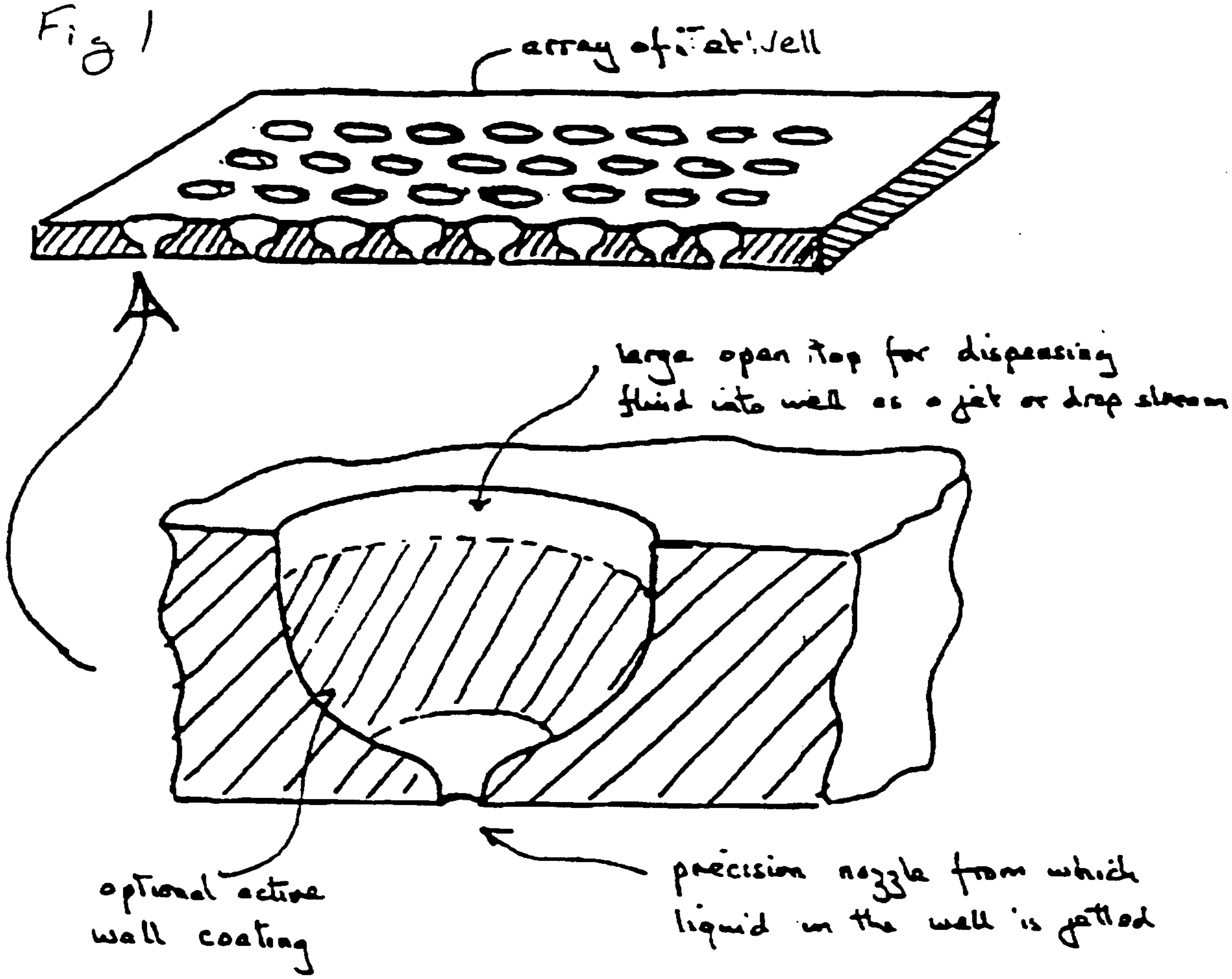

Fig 1A Basic JetWell Structure

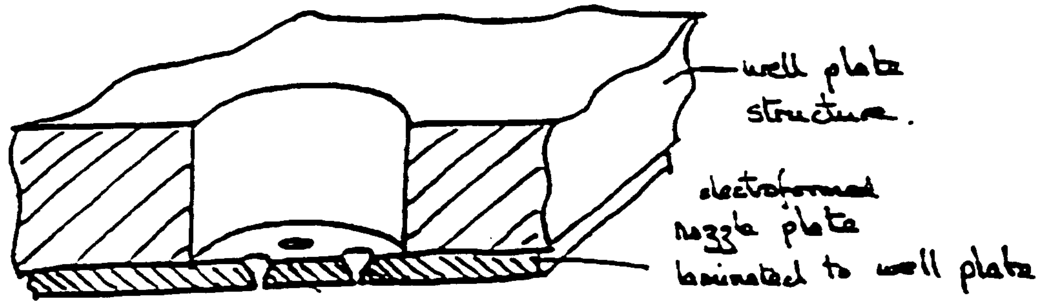

Fig 2a JetWell with multiple nozzles per well.

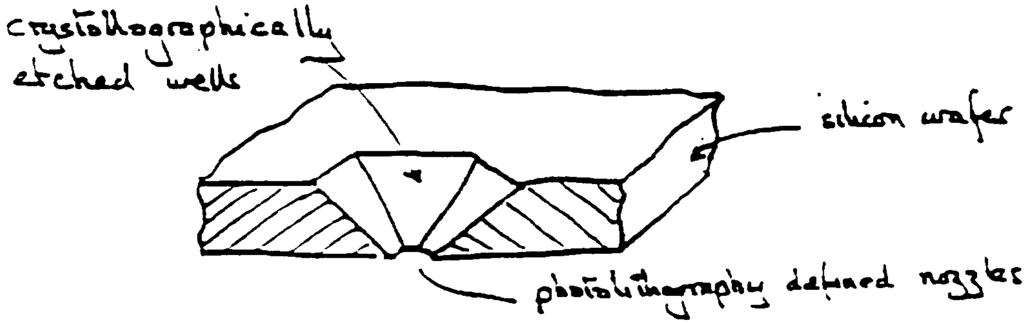

Fig 2b JetWell formed on a silicon wafer

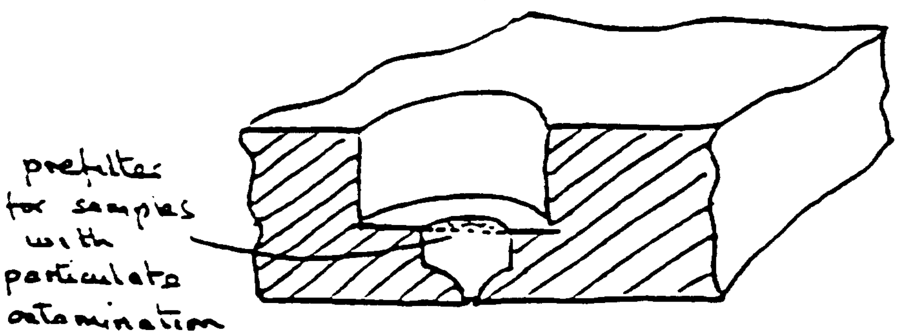

Fig 2c JetWell with integral pre-filter

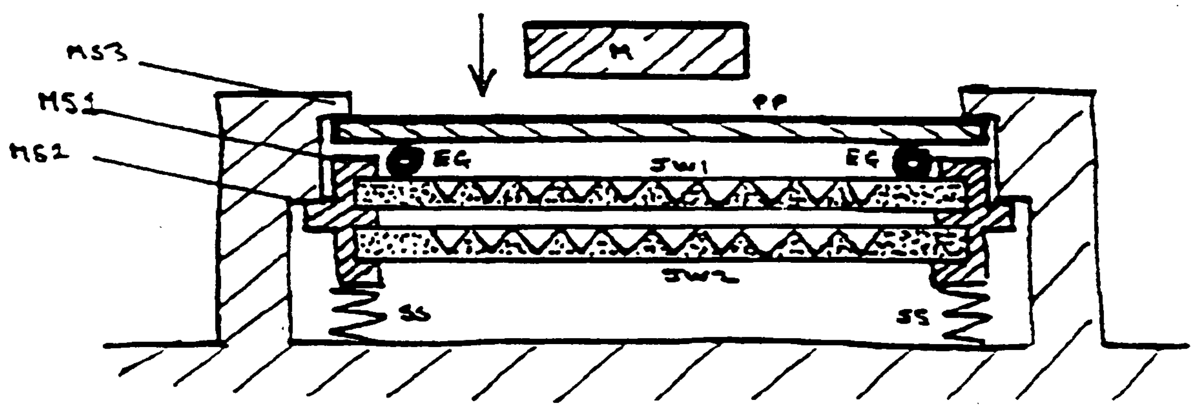

Figure 3 - Impact Actuator

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17072

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01L 3/00; B01L 11/00
US CL :422/102
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/68, 99, 100, 101, 102

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 4,111,754 (PARK) 05 September 1978, see entire document.	1,4 ------- 2-3,5
Y	US 5,395,594 (NOKIHARA et al) 07 March 1995, see entire document.	6-8
Y	US 4,787,988 (BERTONCINI et al) 29 November 1988, see entire document.	6-8
A	US 5,039,493 (OPRANDY) 13 August 1991, see entire document.	1-8
A	US 4,483,925 (NOACK) 20 November 1984, see entire document.	1-8
A	US 4,304,865 (O'BRIEN et al) 08 December 1981, see entire	1-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,493,815 (FERNWOOD et al) 15 January 1985, see entire document.	1-8

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The design of the **JetWell** enables it to receive and deliver accurate micro volumes of fluid.

Dispensing of fluids required for the synthesis into each JetWell would preferably be made without contact of the dispenser to the liquid in the well by using a multinozzle ink-jet head firing into the large end of the wells. A multinozzle approach is necessary to ensure the dispense time for the complete array is short compared to the typical reaction time for each stage of the processing.

Liquid removal from the cells is achieved through the integral nozzle in the bottom of the well. During normal operation liquid is prevented from flowing out the cell through the nozzle by surface tension or even a physical plug. Liquid is ejected from the nozzle by applying pressure to the liquid in the wells causing it to jet cleanly out of the hole away from the lower surface.

The volume to be removed from the well can be controlled by the pressure applied and time for which it is applied. To achieve clean jetting of the fluid pressure typically in the range 0.1 to 10.0 bar gauge is used and the pressure is applied and removed rapidly to prevent any dribbling of liquid out of the nozzle. If a matching JetWell array is positioned beneath the first array transfer of an accurate volume of liquid from one array to the other occurs in a few milliseconds.

The approach provides an accurate system for the parallel dispensing into and from very small JetWell arrays. This enables the volume of the wells to be reduced and the number per plate increased thus greatly increasing the throughput of HTS or CC systems.

This patent application covers the fundamental concept and specific methods of implementing the individual components of the system.

JetWell Array Structure

The specific feature of a JetWell which is different from other forms of microwell plate is that each well has one or more nozzles in the base through which liquid in the well will jet when pressure is applied to the top surface.

The diameter of the nozzle controls the rate of liquid flow and is chosen to optimise for speed and accuracy. For example, if the pressure duration is controlled to 1 millisecond accuracy and the volumetric control requirement is 1 nanolitre the flow rate through the hole must be less than 1 microlitre/second. Assuming a jet velocity of 5 metres/second the nozzle would be around 50 microns in diameter which is comparable with that used in conventional ink jet printers.

The overall volume of each well depends upon the user requirements. A system might be designed to be compatible with conventional 384 well plates which have a well volume around 20 microlitres arranged on a 4.5mm pitch. However, there are considerable advantages in using larger arrays with smaller well volumes.

As an example a plate of similar size to a conventional microwell plate (either 96 or 384) with wells at 1mm pitch would contain a 96 x 64 array. Taking this size as an example the diameter at the top might be 0.8mm and the hole at the base 30 microns in diameter.

Figure 1 shows an example of the basic structure of the JetWell concept and Figure 1A is a detailed view, to an enlarged scale of part of that structure. This application, as well as describing the concept and example JetWell designs also addresses suitable manufacturing techniques for the plate and for the associated system components.

JetWell Plate Manufacture

Where implementations do not require holes below 0.1mm diameter in the base of the wells, conventional moulded manufacturing approaches may be used. However, as smaller holes are required then other approaches will be required. One approach is to use a moulded structure with a thinned section in the base through which a hole is drilled using a laser. Alternatively, a thin sheet with photolithographically defined holes, created

either by etching or electroforming, may be bonded to the bottom of the array.

Another approach is to form each line of wells by bonding two strips together with etched structures in them to form the wells. With this approach the complete array could be formed etched into the surface of a plate which is then cut into strips and laminated together to form the finished structure. Such a structure could be manufactured for example in glass using a low temperature glass enamel bonding.

Where very small wells are required then a single crystal silicon wafer substrate is appropriate with the wells formed by crystallographic etching which can be used to form the nozzle hole with a precision of a few microns.

Examples of these types of construction are shown in Figures 2a, 2b, 2c.

Dispense from the JetWell Plate

The JetWell concept has many wells in an array on a plate each with a nozzle hole in the base. It is possible to transfer a measured dose from all wells on a JetWell plate to a second receiver JetWell plate in one operation. This is achieved by placing the receiver plate directly below the plate containing the liquid to be dispensed and applying a pressure pulse to all wells of the top plate. Liquid is then jetted from each well on the top plate to the corresponding well on the bottom plate. To jet the fluid cleanly from the nozzle hole the pressure in the fluid must rise quickly and fall sufficiently fast to prevent dribbling from the nozzle. The dynamics of this are well understood from ink-jet experience and it is known that it requires rates in excess of 10^8Pa s^{-1} (ie 1 bar/ms) for typical dimensions.

Achieving this rate of pressure rise uniformly over the whole area of the plate is not trivial if contact with the liquid in the wells is to be avoided. One approach to achieving this is to use a piston arrangement over the whole area with the initial distance between the plate and the piston small compared to the lateral dimensions of the plate (eg 5mm spacing for a plate 60 x 40mm). This minimises the volume of air being compressed and the distance

in air for the pressure waves to travel.

The pressure rise of a few bar can then be achieved by moving the piston a few millimetres at speeds of a few metres per second. There are many options for implementing this motion. Where the areas are small, electromagnetic actuators are the simplest. However, for a plate area of 100 x 100 mm the force required to reach 10 bar is 10^4Newton's (~ 1 ton) which is more difficult to achieve using a simple solenoid drives.

To meet this requirement an impact actuator can be used. In this approach, the piston is sealed to the dispense JetWell plate using a compliant seal which permits sufficient travel to reach the desired pressure and then prevents over pressure by limiting the motion using a physical stop. The receiver JetWell plate is fitted directly below the dispense plate. The piston and the two JetWell plates are mounted together on a spring support. The back of the piston is then struck by a mass at a predetermined velocity. The mass striking the piston back moves the piston towards the plate until it reaches the stop. This compresses the air volume above the wells to the desired pressure very quickly. Both the impact mass and the plates continue to move compressing the support spring. The spring decelerates the assembly and then accelerates it back-up. Both JetWell plates are stopped at the original position but the piston is free to continue. As the piston and impact mass move away from the plates the pressure above the wells falls rapidly. The piston and impact mass are then decelerated, using viscous damping, to a stop. This apparatus is illustrated, by way of example, in Figure 3. It should be noted that there is a temperature rise associated with the rapid air compression which must be accounted for in the design.

Figure 3 shows two JetWell plates, each of the type shown in Figure 1, mounted in the impact actuator. JWL is the JetWell plate containing the liquid to be dispensed and JW2 is the JetWell plate into which the aliquots are to be dispensed. JW1 and JW2 are mounted rigidly together with JW1 above JW2. The piston plate PP is mounted with a small gap above JW1 on a compressible gas tight edge gasket EG. JW1, JW2 and PP are all supported on spring support SS.

In use, mass M is accelerated towards PP so that it impacts at a specified velocity. The piston plate PP and mass M move towards JW1 compressing the edge gasket (EG) until the motion stop (MS1) stops it at a preset gap. This raises the pressure rapidly and uniformly over all of JW1 to the pressure required to jet the liquid from the nozzles in the base of the wells. M, PP, JW1 and JW2 all continue to move down compressing spring support SS. The spring support SS oscillates forcing M, PP, JW1 and JW2 upwards after a time governed by resonant frequency of the spring/mass system. Upward motion of JW1 and JW2 is halted by the stop MS2 with PP and M continuing upwards by their inertia. PP and M are stopped by the stop MS3 at their starting position so that the pressure above JW1 returns rapidly to 1 bar.

The dispense pressure is a function of air volume and the distance to the forward stop and the dispense time is a function of the spring constants, masses and initial impact velocity. The technique therefore provides the ability to independently set time and pressure for dispensing over a wide range.

Where dispensing is required from a single well only then a small area piston pump can be located over the specific well. Similarly lines, or rectangular sections of the plate can be addressed by specifically shaped actuators. The use of a minimal volume positive displacement air pressure generator has important advantages as has the impact technique described to implement it.

<u>Dispense to the JetWell Plate</u>

The technique for dispensing from a JetWell plate can also be used for dispensing to a JetWell plate providing the same volume is required in each well. This is applicable in some, but not all instances. Where different amounts are required in each well, alternative approaches can be used.

Several approaches for this have been developed depending upon specific requirements.

One approach is to use the minimal volume pressure generator approach described previously with a patterned nozzle plate which addresses only those wells which are to receive the dose. This can be implemented by:

* Pre-fabricating a nozzle plate having the desired pattern of nozzles which is fitted to the dispenser when required. Nozzle plates could be selected from a library and cleaned for re-use

* A programmable nozzle array.

There are many possible implementations of programmable nozzle arrays including but not restricted to solenoid, piezo, mechanically and thermal drives for ball, plate, disc or poppet mechanisms.

However, implementation for nozzles in the sub 0.1mm diameter range with minimal dead volume and implementable in arrays of many thousand is a challenge. One approach is to use cone shaped nozzles which are produced as an array in a ferro magnetic material. The nozzle plate is removable from the pressure generating head. To programme the array to the required pattern, magnetic beads are placed in those nozzles to be closed. The magnetic attraction to the plate holds them in place whilst the plate is replaced in the dispense head. The nozzles are then filled with the liquid to be dispensed and a pressure pulse applied for the required time. The ball in cone arrangement ensures a good seal against the dispense pressure.

After operation the liquid is removed and the nozzle plate back-flushed to remove the beads before being cleaned for re-use. The magnetic beads may be disposable or reusable depending upon application. By using several plates which can be programmed whilst other operations are being performed the system will be rapid and flexible.

In other cases it may be necessary to dispense different volumes of a range of liquids to the wells. This can be achieved using the JetWell system in a pre-fill capacity. In this approach the JetWells of the dispense plate are prefilled with the volume and type of

liquids required using an accurate but not necessarily fast technique. The liquid is held in the wells by surface tension. When all wells have been filled with the predetermined volume of the appropriate liquid, the plate is ready for use. It is positioned over the reaction JetWell plate, and all the content of each well is jet dispensed by a pressure pulse as described previously.

This approach is most appropriate where it is necessary to make the transfer into the wells quickly but there is time between transfers to pre-load the dispenser. Care must be taken to avoid solvent loss by evaporation from the wells between filling and dispensing.

In some cases neither of these JetWell dispensing approaches will be appropriate. For these instances it may be necessary to use a precision dispensing head. The technique most suitable for this is based upon drop-on-demand ink-jet systems where the dose in each well can be set by dispensing a measured number of drops.

The most rapid dispensing would be achieved by having a two dimensional array of nozzles to match the plate. However, this is difficult to construct and hence expensive. The preferred approach is to use a linear array of nozzles which is scanned across the plate. Commercially available ink-jet heads are unlikely to be appropriate, mainly because of the pitch between nozzles and their choice of materials, and a specific implementation is therefore needed. The features of the head which are required are:

* The dead volume of the system must be minimised to avoid wastage of reagents which in some cases may be expensive

* All material in contact with the fluid must be inert to a wide range of acids, alkalis and solvents. Preferably it should be restricted to silicon dioxide, stainless steel, and inert polymers

* The system must be easy to fill and to flush as it must be used many times with a wide range of fluids

* The nozzle diameter is chosen to match the well size. Typically the drop size is selected to be in the range 10^{-3} to 10^{-4} of the well volume

* Each nozzle is operated independently in a drop on demand mode. Typical delivery rates are in the range 10^3 to 10^4 drops per second

* Actuation will preferably be by a thin piezo layer bonded to a silicon or silicon dioxide membrane.

For high density arrays the piezo drivers may be integrated on to the head. Alternatively the head would have gold contacts deposited enabling it to make electrical contact to off head drivers.

The application lends itself to implementation of the head using silicon crystallographic etching techniques to form the structure and either silicon to glass or silicon to silicon bonding for assembly.

Either top shooter or side shooter forms could be implemented at typical pitches required ($>$0.5mm).

Calibration

The precision of dispensing achievable either by pressurised jetting or by drop on demand devices depends upon the accurate control of a range of parameters. Where the nozzles become very small it is difficult to achieve precision from the accuracy of manufacture alone. However, by reasonable control of the operating parameters reproducibility can be excellent.

The best approach for achieving precision is therefore to include a technique for the calibration of each device prior to use. Thus, for example, for JetWell plates there may be a distribution of nozzle diameters coupled with a pressure variation which leads to different volumes being dispensed for each well. If a quantative assay is being performed

on part-samples from each well, volume variation would influence the result.

The system could be calibrated before use by dispensing a reagent which can be used in a simple standard assay test for volume. This for example could be a colour change reaction which is convenient for automated measurement.

The calibration would be used to correct the results of the real assay rather than to control the volumes dispensed.

The implementation of automated calibration is an important technique for achieving high precision for multinozzle microdispensing systems.

CLAIMS

1. A multiwell plate where the wells have a large opening at the top and a small nozzle hole in the base.

2. A multiwell plate where the diameter of the small hole in the base is chosen so that a jet of liquid is emitted when a pressure pulse is applied to the surface such that by selecting a time for the pressure pulse a precise amount of the volume in the well can be dispensed.

3. A multiwell plate as in (1) which has immobilisation coating on the walls for the purpose of synthesis or assay.

4. A multiwell plate as in (1) in which the nozzle is fabricated by moulding, laser drilling, photolithographic processes, or by etching.

5. A multiwell plate according to claim 1 which is fabricated by stacking layers together which have etched structures which form the wells.

6. The use of an air pressure to eject measured volumes of liquid from the nozzles in the base of the wells described in (1).

7. The generation of the air pressure pulse by the movement of a piston plate in close proximity to the surface of the multiwell plate.

8. The generation of the movement of the piston used in (6) by electrical, hydraulic or mechanical impact means.

FIG. 1

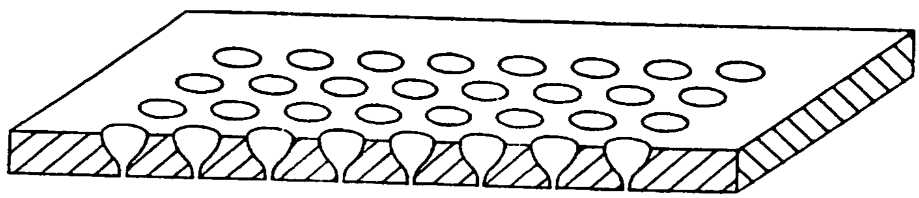
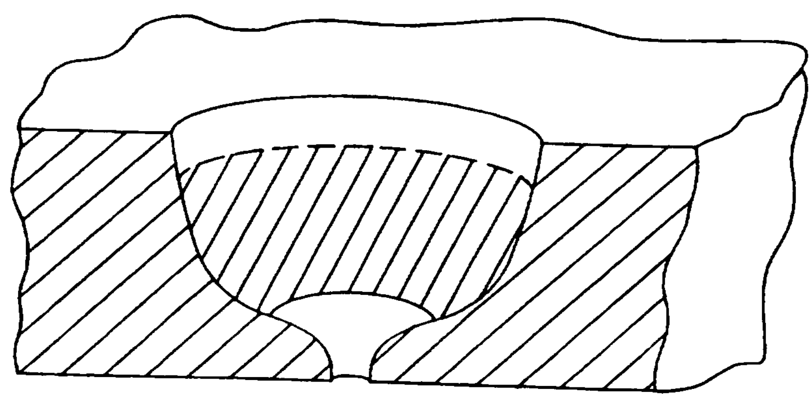


FIG. 1A



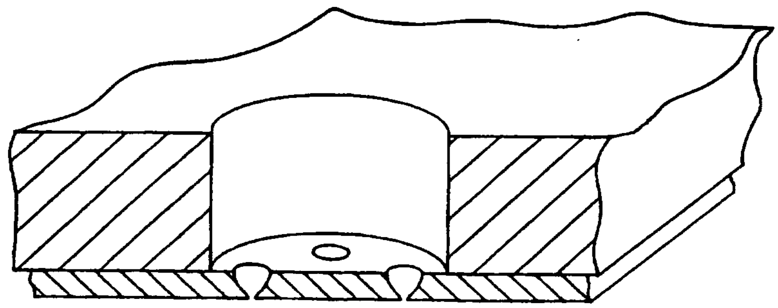


FIG. 2A

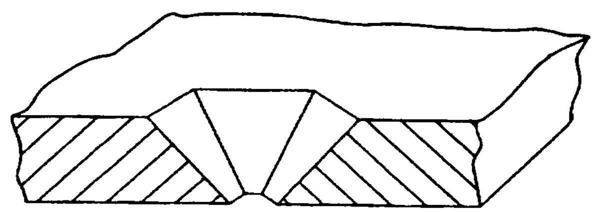


FIG. 2B

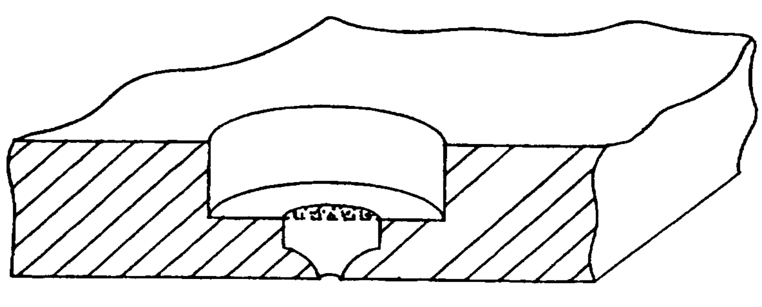


FIG. 2C

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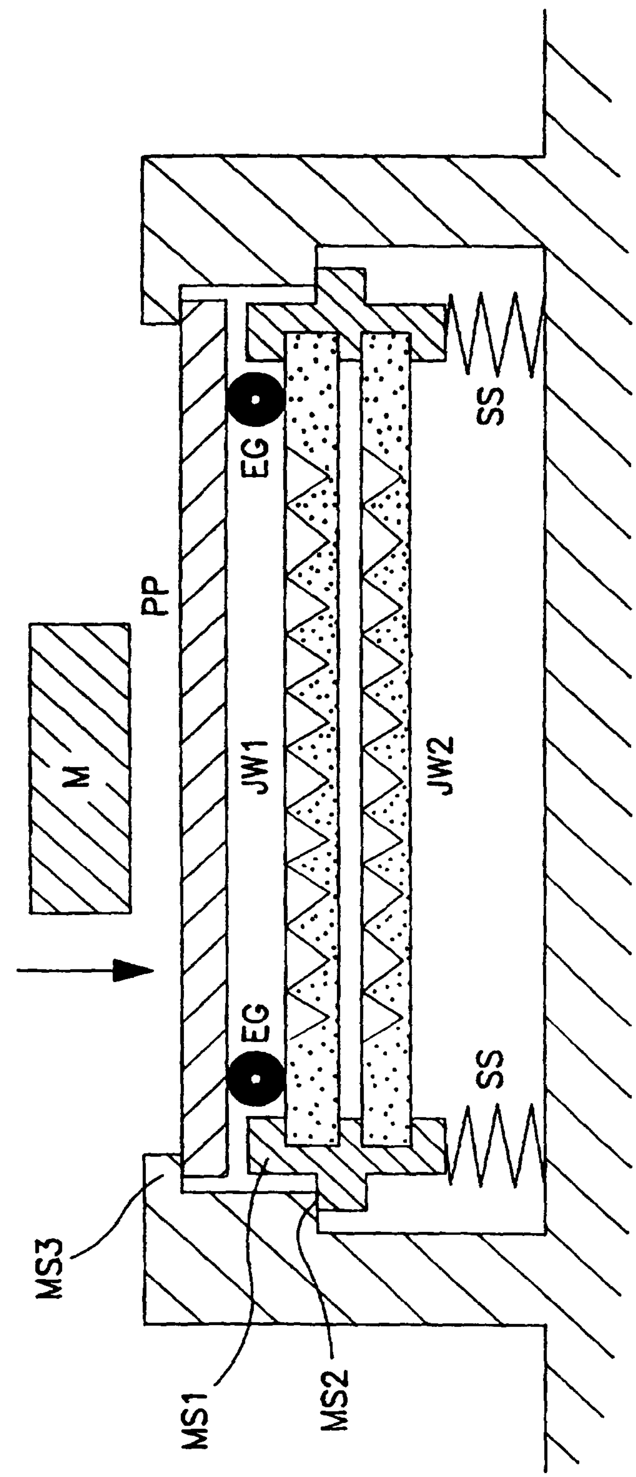

FIG. 3